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Dinemasporium japonicum on *Polytrichum commune* from Korea

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Abstract — *Dinemasporium japonicum* was found growing on the decaying stems of *Polytrichum commune* (*Bryophyta*). The identification of *D. japonicum* was based on its morphological characteristics and the sequence analysis of the internal transcribed spacer of ribosomal DNA. This represents the first record of *D. japonicum* on a non-tracheophyte substrate, and the first record of its occurrence in Korea.

Key words — common haircap moss, ITS rDNA, phylogeny, *Polytrichaceae*

Introduction

Dinemasporium Lév. (*Chaetosphaeriaceae*, *Sordariomycetes*) is characterized by setose conidiomata exposing a slimy conidial mass at maturity, phialidic conidiogenous cells, and hyaline, oblong to allantoid, aseptate conidia with an appendage at each end (Crous et al. 2012; Hashimoto et al. 2015). It is a phylogenetically well-defined genus in the *Chaetosphaeriaceae*, and morphological characteristics such as conidiomatal setae, conidial size, and length of conidial appendages are useful for distinguishing its species (Crous et al. 2012).

In June 2012, mats of common haircap moss (*Polytrichum commune* Hedw., *Polytrichaceae*, *Bryophyta*) were found to be dotted with brown to

purplish brown patches on Mt. Cheongtae located in Hoengseong county in Korea. Careful observation of the discoloration revealed that some of the moss stems and leaves were associated with fungal growth. Decaying mosses were characterized by conidiomata with setae large enough to be visible to the naked eye. A short survey showed that hundreds of the moss plants were decayed by one type of fungus. Here, we identify this fungus as *Dinemasporium japonicum*, based on morphological characteristics and phylogenetic analyses.

Materials & methods

Fungal structures were detached from the moss and placed on a glass slide in a drop of water. Observations were made with an Olympus BX51 microscope (Olympus, Tokyo, Japan) and with a Zeiss AX10 microscope using bright field and differential interference contrast (DIC) light microscopy and an AxioCam MRc5 camera (Carl Zeiss, Göttingen, Germany). At least 30 measurements of each fungal structure were conducted at 100–1000×. A voucher specimen was deposited in the Korea University Herbarium, Seoul, Korea (KUS).

To obtain a pure isolate, the fungal structures with a conidial mass were carefully removed and placed in a drop of sterilized water on a glass slide, since conidial dispersal occurs in water. A loop of conidial suspension was streaked onto 2% water agar plates supplemented with streptomycin sulfate (100 mg/L). After 1–2 days of incubation at 25°C, the conidia began to germinate. Single conidial colonies were transferred onto potato dextrose agar (PDA) using a fine needle. The obtained isolate was deposited in the Korean Agricultural Culture Collection, Wanju, Korea (KACC).

Genomic DNA was extracted from fungal colonies growing on PDA using DNeasy Plant Mini DNA Extraction Kits (Qiagen Inc., Valencia, CA, USA), following the manufacturer's instructions. The complete internal transcribed spacer (ITS) region of rDNA was amplified with the primers ITS1/ITS4 (White et al. 1990) and purified using a QIAquick PCR Purification Kit (Qiagen Inc.). The purified PCR amplicons were directly sequenced using Macrogen Sequencing Service (Macrogen, Seoul, Korea). The newly generated sequence was deposited in GenBank. Several ITS rDNA sequences of *Dinemasporium* species in the NCBI GenBank database were retrieved for the phylogenetic analysis. *Pseudolachnea hispidula* was used as outgroup. Sequences were aligned using MAFFT version 7 (Katoh & Standley 2013). A phylogenetic tree was inferred using the neighbor-joining method in MEGA6 (Tamura et al. 2013), with the default settings of the program. The reproducibility of the internal nodes in the tree was tested by bootstrap analysis using 1000 replications.

Taxonomy

Dinemasporium japonicum A. Hashim., G. Sato & Kaz. Tanaka, Mycoscience 56: 92. 2015 ["2014"]. Fig. 1

Fungal colonies on PDA were flat, ivory to pale yellow, floccose, with sparse aerial mycelium. Conidiomata were stromatic, scattered or aggregated,

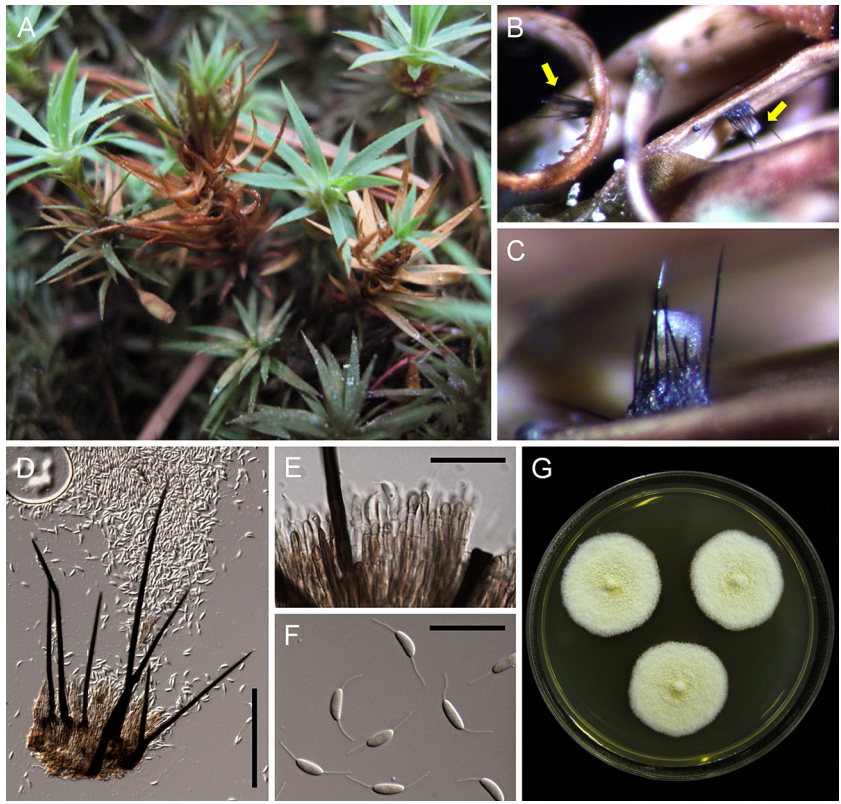

Fig. 1. *Dinemasporium japonicum* on *Polytrichum commune*. A: Discoloration of stems and leaves of the common haircap moss associated with fungal growth. B: Close-up view of leaves, showing fungal structures (arrows). C, D: Conidioma with setae, containing a conidial mass. E: Conidiophores with conidiogenous cells. F: Conidia with two appendages. G: Two-week-old colonies on potato dextrose agar. Scale bars: D = 100 µm; E, F = 20 µm.

superficial, pulvinate, dark brown to black, setose with pale brown conidial mass in center. Setae were mostly marginal or occasionally in conidiomata, stiff, straight, unbranched, dark brown to chestnut brown, pointed, 1–7-septate, attenuate upwards, 70–300 µm long, and 4–12 µm wide at the base. Conidiophores were lining the basal stroma, cylindrical, hyaline, septate, thin-walled, invested in mucus, 22–36 µm long, and 2–3 µm wide. Conidiogenous cells were phialidic, determinate, subcylindrical to lageniform, hyaline, 5–12 µm long, and 2–2.5 µm wide. Conidia were fusiform to allantoid or naviculate, obtuse on both ends, unicellular, hyaline, milky white to pale pinkish in a slimy

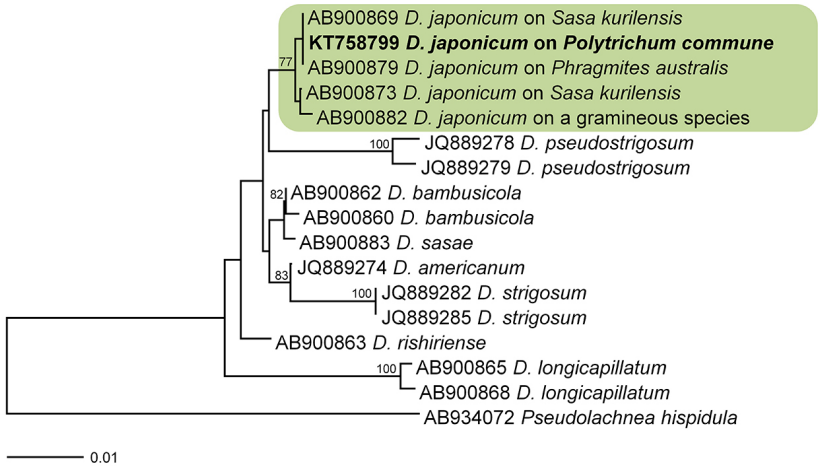

Fig. 2. ITS phylogenetic tree inferred from the neighbor-joining method for *Dinemasporium japonicum* and allied taxa, based on the sequences of the internal transcribed spacer rDNA region. Bootstrap support values from 1000 replicates are shown at the nodes. Our new sequence is indicated by bold font. Scale bar indicates number of nucleotide substitutions per site.

mass, and 6.5–9 µm long, and 2–3 µm wide. Appendages were 7–11 µm long and almost centric.

Specimen examined – **KOREA**, **Gangwon**, Hoengseong, Mt. Cheong Tae, 37°31′19″N 128°17′25″E, on the decaying stems of *Polytrichum commune*, 18 June 2012, H.D. Shin & J.H. Park (KUS-F26722; culture, KACC46848; GenBank KT758799).

Remarks — The lengths of the conidiophores and conidiogenous cells in our study differed somewhat from those given in the original description (conidiophores 4–17 µm, conidiogenous cells 6–21 µm; Hashimoto et al. 2015). In *Dinemasporium* species, the conidiophores are generally much longer than the conidiogenous cells (Duan et al. 2007; Crous et al. 2012). The holotype specimen of *D. japonicum* should be reexamined to clarify the unusual description for this species.

Phylogeny

The sequence obtained from KACC46848 comprised 502 bp. A BLAST search of this sequence revealed 100% identity with sequence AB900869 from the holotype of *D. japonicum* (HHUF30103). Based on the ITS rDNA sequences,

our Korean sequence clustered within the clade of *D. japonicum*, distinct from other *Dinemasporium* species in the neighbor-joining tree (Fig. 2). Previously, the ITS region provided insufficient resolution for the interspecific relationships of most *Dinemasporium* species. In our study and in that of Hashimoto et al. (2015), however, the ITS region clearly discriminates *D. japonicum* from other *Dinemasporium* species and supports it as a separate lineage.

Discussion

Recently, Hashimoto et al. (2015) introduced *D. japonicum* as a new species occurring on some gramineous (*Juncus*, *Miscanthus*, *Phragmites*, *Sasa*) and unidentified woody substrates from Japan. In general, *Dinemasporium* species occur on various herbaceous and woody substrates (Nag Raj 1993); however, they have never been reported on bryophytes. Our Korean specimen represents the first observation of *D. japonicum* on a non-tracheophyte substrate, and the first record of this fungus in Korea.

Two species, *Epigloea pleiospora* in Poland and *Lizonia emperigonia* in Denmark, Poland, and Sweden, have been recorded on *Polytrichum commune* as phytopathogens, and several *Dinemasporium* species have been reported as phytopathogens on various vascular plants (Farr & Rossman 2016). Although we could not evaluate the phytopathological effects of *D. japonicum* on *P. commune*, this fungus is certainly associated with the discoloration and decomposition of the moss.

Acknowledgements

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